

**GLADSTONE ROAD AGRICULTURAL CENTRE
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**WEANING AND HARDENING OF TISSUE CULTURED BANANA, CASSAVA AND SWEET
POTATO PLANTLETS**

Kenneth VA Richardson
Department of Agriculture
Nassau, Bahamas
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The Orange Hill Tissue Culture Laboratory, St. Vincent and the Grenadines

Introduction

This paper is based on a regional training workshop entitled ‘Tissue Culture Protocols and Weaning and Hardening of Plantlets of Root and Tuber Crops’ conducted at the Orange Hill Tissue Culture Laboratory, St. Vincent and the Grenadines, from 25-26 March 2015. This workshop brought together more than a dozen Caribbean scientists from the region to demonstrate the protocols for plant tissue culture and to train them in the correct procedures for the weaning and hardening of such material. The Orange Hill Tissue Culture Laboratory of St. Vincent and the Grenadines serves as a training centre and facility for the large-scale production of plant species, in particular root and tuber crops, bananas, pineapples and other important tropical crops. The centre also serves as a germplasm bank for the Caribbean region.



Hardening facility at the Orange Hill Tissue Culture Laboratory

Overview of Plant Tissue Culture

Plant tissue culture is the technique of generating whole plants from single cells, tissues or organs, under controlled conditions and in a sterile environment. The cells, tissues or organs are grown on an artificial medium that supplies nutrients required for growth and development.



Hardening Procedure for Sweet Potato

For the hardening procedures for the sweet potato, the medium consists of shredded coconut, sifted to remove any large particles. The sifted coconut fibre is placed in plastic trays and dampened with water. A commercial potting soil medium may be used instead of the shredded coconut. The tissue culture containers are dipped in water for about thirty seconds after which the plantlets are gently removed. The plantlets are immersed in water to remove the agar medium, with care being taken not to damage the delicate roots. The plantlets are then carefully placed into the tray's cells, and then watered.



Preparation for hardening of sweet potato in the shade house.



Plantlets are removed gently from tissue culture container



Sweet potato plantlets immersed in water to remove the agar medium.



Freshly planted sweet potato in trays. Note the small holes in tray on the right, ready for planting.

Hardening Procedure for Cassava

The cassava plantlets are left in their tissue culture containers for at least two days before being transferred to the growing medium. The medium used in the practical experience consisted of a mixture of river sand, treated with hot water, and shredded coconut fibre, in a one to one ratio (substitute with a potting soil mixture). Each plantlet is transplanted individually to small polystyrene cups with holes at the bottom for drainage, then placed in a water bath to which is dissolved a complete fertiliser, with high phosphorus content. The plantlets are covered with a polystyrene cup for about two days. After two days the covers are removed and the plantlets placed under shade of about fifty percent. After a further three weeks, the cassava plantlets are ready for transplanting directly to the field.



Preparation of soil mixture for cassava plantlets.



Preparation of polystyrene cups for weaning of cassava plantlets.



Single cassava plantlet, removed from tissue culture container.



Cassava plantlets covered with polystyrene cups and placed in a water bath containing high phosphorus fertiliser.

Hardening Procedure for Banana

The procedure for the hardening of tissue cultured banana plantlets is divided into two stages. The first stage begins while the plantlets are still growing in the tissue culture containers. Though the banana plantlets have roots and shoots, they are not able to survive in the outdoors. They are weaned by removing the container tops and exposing them gradually to more light over a ten-day period. The agar is removed by immersing the banana plantlets in water. The plantlets are then transferred to trays containing the appropriate soil medium and watered thoroughly. After about one week a slow release fertiliser is applied to the plantlets. The plantlets remain in

the trays for about four to six weeks, during which time they are treated with a foliar fertiliser and sprayed for insect pests.



First stage hardening of banana tissue cultured plantlets

After about four to six weeks, the plantlets are ready for the second stage of hardening. In the second stage of hardening, the plantlets are transferred to individual polyethylene bags or plastic pots. The potting mixture may consist of sand mixed with clean soil, or a commercial potting mix. The plants are then transferred to a shade house that provides about fifty percent shade and roofing that allows rain to penetrate, as the plantlets require lots of moisture for proper growth and development. The plantlets should be irrigated periodically to avoid them drying out. Fertiliser with high phosphorus content should be administered in two applications, after two and four weeks of growth. The banana plantlets are ready for transplanting to the field after about six to eight weeks in the shade house.



Second stage hardening of banana plantlets

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